CLAIMS

1. A method of generating T-cell lines and clones specific to neisserial proteins, the method comprising isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with neisserial proteins with or without a proliferation stimulant for a prescribed period, stimulating proliferation of T-cell lines and clones which are specific to neisserial proteins, and maintaining same by regular stimulation.

2. A method as claimed in claim 1, characterised in that the neisserial proteins are prepared from *Neisseria meningitidis* and/or *Neisseria gonorrhoea* grown under iron restrictions to induce the expression of iron-regulated proteins.

3. A method as claimed in ~~any preceding claim~~ claim 1, characterised in that the peripheral blood is obtained from naturally infected patients at different stages of illness.

~~4. A method as claimed in claim 3, characterised in that the stages include an acute stage (on admission), early convalescence (seven days after admission), late convalescence (six weeks after discharge) and after full recovery (3 months and twelve months after discharge).~~

5. A method as claimed in ~~any preceding claim~~ claim 1, characterised in that the peripheral blood is heparinised or treated with ESTA.

6. A method as claimed in ~~any preceding claim~~ claim 1, characterised in that the PBMCs are isolated from the blood by centrifugation.

7. A method as claimed in ~~any preceding claim~~ claim 1, characterised in that the PBMCs are initially cultured in medium containing human serum.

a 8. A method as claimed in claim 1 ~~any preceding claim,~~ characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period.

Sub a2 9. A method as claimed in claim 8, characterised in that the predetermined period is 3-10 days and may be 5 days.

10. A method as claimed in any of claims 8 or 9, characterised in that IL-2 stimulates the proliferation of the activated T-cell lines and clones.

11. A method as claimed in claim 10, characterised in that the T-cell lines and clones are maintained by weekly stimulation.

12. A method as claimed in claim 10 or claim 11, characterised in that the stimulation is provided by proteins in the presence of IL-2 and feeder cells.

13. A method as claimed in claim 12, characterised in that the feeder cells are antigen presenting feeder cells and may be autologous Epstein-Barr virus transformed B-lymphocytes (EBVB).

a 14. A method as claimed in claim 1 ~~any preceding claim,~~ characterised in that the specificity of the T-cell lines and clones to neisserial proteins is tested prior to storing for example in liquid nitrogen.

Sub a3 15. A method as claimed in claim 14, characterised in that the specificity is tested by measurement of tritiated thymidine incorporation in response to stimulation with neisserial proteins compared to irrelevant antigens.

16. A method as claimed in claim 15, characterised in that an irrelevant antigen is tetanus toxoid.

a 17. A method as claimed in claim 1 ~~any preceding claim~~, characterised in that a ~~the~~
a phenotype of the T-cell lines and clones are ~~also~~ assessed using flow cytometry

and specific monoclonal antibodies.

18. A method as claimed in claim 17, characterised in that the antibodies are CD4+, CD8- and α/β- and γ/δ- T-cell receptor (TCR) specific monoclonal antibodies.

19. A method of detecting CD4+ T-cell stimulating proteins, the method comprising fractionating neisserial proteins and testing the ability of said proteins to stimulate proliferation of Neisseria specific T-cell lines and clones generated according to the method as claimed in any of the preceding claims.

20. A method as claimed in claim 19, characterised in that the proteins are fractionated by SDS-PAGE.

21. A method as claimed in any of claims 19 or 20, characterised in that the fractions are tested for their ability to stimulate the individual T-cell lines and clones.

22. A method as claimed in claim 21 19, characterised in that fractions containing T-cell stimulants are further characterised by SDS-PAGE.

23. A method as claimed in claim 19 any of claims 19 to 22, characterised in that polyclonal antibodies are raised to the T-cell stimulating fraction proteins.

24. A method as claimed in claim 23, characterised in that the antibodies are used to screen a genomic meningococcal and/or gonococcal expression library.

25. A method as claimed in claim 24, characterised in that the expression library is a λZapII library.

26. A method as claimed in claim 24 or claim 25, characterised in that isolated neisserial polypeptides which react with the antibodies and their respective DNA fragments are further characterised and sequenced.

27. A method of detecting ~~CD4⁺ T-cell stimulating recombinant proteins, the~~ method comprising screening a genomic meningococcal or gonococcal expression library for recombinant proteins which stimulate T-cell lines and clones.

28. A method as claimed in claim 27, characterised in that the T-cell lines and clones are meningococcal and/or gonococcal specific T-cell lines and clones ~~generated according to the method of any of claims 1 to 18~~.

9. A method as claimed in claim 27 ~~or claim 28~~, characterised in that the enomic meningococcal or gonococcal expression library is a λZapII phage ibrary expressing genomic DNA extracted from a strain of *Neisseria neningitidis* or a strain of *Neisseria gonorrhoea*.

10. A method as claimed in claim 29, characterised in that a representative ool of recombinant pBluescript SKII plasmid are excised from the phage ibrary and transformed into *E.coli* strain XL1-Blue.

11. A method as claimed in claim 30, characterised in that the plasmids are xcised into XL1-Blue using a helper phage.

32. A method as claimed in claim 30 or claim 31, characterised in that the transformed *E.coli* are cultured in a medium which may contain ampicillin.

33. A method as claimed in claim 27 ~~any of claims 27 to 32~~, characterised in that meningococcal or gonococcal protein expression is induced by isopropyl-b-D-thio-galactoside.

34. A method as claimed in claim 27 ~~any of claims 27 to 33~~, characterised in that the bacteria are heat-killed and sonicated before adding to antigen presenting cells.

35. A method as claimed in claim 27 ~~any of claims 27 to 34~~, characterised in that the expressed proteins are tested for their ability to stimulate the individual T-cell

lines and clones.

36. A method as claimed in ~~any of claims 27 to 35~~ claim 27, characterised in that CD4+ T-cell stimulating bacterial cultures are identified and subcultured.

37. A method as claimed in claim 36, characterised in that the subcultures are preferably rescreened for T-cell stimulation.

38. A method as claimed in claim 36 or claim 37, characterised in that the CD4+ T-cell stimulants are identified by sequencing and are further characterised.

39. A method as claimed in any of claims 27 or 28, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a meningococcal or gonococcal genomic lambda phage display library.

40. A method of detecting CD4+ T-cell stimulating peptides, the method comprising screening meningococcal or gonococcal genomic phage display libraries (PDLs) to identify peptides which stimulate T-cell lines and clones.

41. A method as claimed in claim 40, characterised in that the T-cell lines and clones are meningococcal and/or gonococcal specific T-cell lines and clones ~~generated according to the method as claimed in any of claims 1 to 18~~.

42. A method as claimed in ~~any of claims 39 to 41~~ claim 40, characterised in that the genomic phage display library (PDL) is generated by fragmenting bacterial DNA, cloning and packaging into bacteriophage vectors.

43. A method as claimed in claim 42, characterised in that two vectors are used.

44. A method as claimed in claim 43, characterised in that the first vector

displays peptides up to 1200 amino acids which are expressed at low copy numbers.

45. A method as claimed in claim 43 or claim 44, characterised in that the second vector preferably displays up to 415 copies of a peptide up to 50 amino acids in size.

46. A method as claimed in ~~any of claims 40 to 45~~ claim 40, characterised in that the PDLs are amplified in respective *E.coli* hosts.

47. A method as claimed in ~~any of claims 40 to 46~~ claim 40, characterised in that the cells are heat killed before testing for the ability of the peptides to stimulate the T-cell lines and clones.

48. A method as claimed in ~~any of claims 40 to 47~~ claim 40, characterised in that $CD4^+$ T-cell stimulating PDL cultures are identified and subcultured.

Sub 49 49. A method as claimed in claim 48, characterised in that the subcultures are rescreened for T-cell stimulation.

50. A method as claimed in ~~any of claims 40 to 49~~ claim 40, characterised in that the $CD4^+$ T-cell stimulants are identified by sequencing and are further characterised.

51. A method of detecting $CD4^+$ T-cell stimulating recombinant proteins, using a meningococcal or gonococcal genomic lambda phage display library ~~in accordance with any of claims 27 to 39~~.

52. A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a pre-digested vector, and plating by infecting *E.coli*.

53. A method as claimed in claim 52, characterised in that the vector is a lambda phage.

54. A method as claimed in claim 53, characterised in that the vector is λpRH825 vector.

55. A method as claimed in claim 53 or 54, characterised in that the amplified and digested DNA fragments are packaged into the lambda phage using a lambda phage packaging kit.

56. A method as claimed in any of claims 52 to 55, characterised in that the restriction sites are SpeI or NotI.

57. A method as claimed in any of claims 51 to 56, characterised in that the DNA inserts in the plaques formed are sequenced, thereby confirming that the plaques contain DNA fragments of meningococcal or gonococcal origin.

58. Use of a polypeptide in the manufacture of a vaccine against neisserial disease, the peptide comprising an amino acid sequence as shown in SEQIDNO1 and SEQIDNO2 or an active derivative thereof.

59. A polypeptide as claimed in claim 58, characterised in that the polypeptide is a $CD4^+$ T-cell stimulant.

60. A DNA construct for use in the manufacture of a medicament for the treatment of neisserial disease the construct comprising a sequence as shown in SEQIDNO3 or an active derivative thereof.

61. Use of a polypeptide in the manufacture of a vaccine against neisserial disease, the peptide comprising an amino acid sequence as shown in SEQIDNO3 and SEQIDNO4 or an active derivative thereof.

62. A polypeptide as claimed in claim 61, characterised in that the

polypeptide is a CD4+ T-cell stimulant.

63. A DNA construct for use in the manufacture of a medicament for the treatment of neisserial disease, the construct comprising a sequence as shown in SEQIDNO1, or an active derivative thereof.

64. A composition for use as a vaccine against neisserial disease, the composition comprising two peptides with the amino acid sequences as shown in SEQIDNO1 and SEQIDNO2, and SEQIDNO3 and SEQIDNO4 or active derivatives thereof.

65. A nucleotide sequence comprising a base sequence as shown in SEQIDNO1, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQIDNO1 and SEQIDNO2, or an active derivative thereof.

66. A nucleotide sequence comprising a base sequence as shown in SEQIDNO3, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQIDNO3 and SEQIDNO4, or an active derivative thereof.

67. A vaccine against neisserial disease, the vaccine comprising polypeptide with the amino acid sequence as shown in SEQIDNO2 or an active derivative thereof.

68. A vaccine against neisserial disease, the vaccine comprising polypeptide with the amino acid sequence as shown in SEQIDNO4 or an active derivative thereof.

69. A method of treatment of neisserial disease, the method comprising inducing T-cell proliferation with polypeptide comprising one or both of the amino acid sequences shown in SEQIDNO2 and SEQIDNO4, or active derivative(s) thereof.

70. A purified and isolated DNA composite comprising the sequence shown in SEQIDNO1, or an active derivative thereof.

71. A purified and isolated DNA composition comprising the sequence shown in SEQIDNO3, or an active derivative thereof.

72. A methodology substantially as hereinbefore described with reference to the accompany drawings and sequences.

73. Use of a polypeptide substantially as hereinbefore described with reference to the accompany drawings and sequences.

74. A DNA construct substantially as hereinbefore described with reference to the accompany drawings and sequences.

75. A composition substantially as hereinbefore described with reference to the accompany drawings and sequences.

76. A nucleotide sequence substantially as hereinbefore described with reference to the accompany drawings and sequences.

77. A vaccine substantially as hereinbefore described with reference to the accompany drawings and sequences.

78. A method of treatment substantially as hereinbefore described with reference to the accompany drawings and sequences.

79. Any novel subject matter or combination including novel subject matter disclosed herein, whether or not within the scope of or relating to the same invention as any of the preceding claims.